REMARKS

Claims 27-36, 38-47, 49-59 and 61-68 are pending, applicants having cancelled claims 37, 48 and 60 in applicants' last amendment.

Applicants thank the Examiner for withdrawing prior 35 U.S.C. § 112 ¶1 and ¶2-based rejections, and the 35 U.S.C. § 103(a)-based rejection over Herman et al. (U.S. Patent 6,017,704).

The 'new matter' rejection of claims 33, 35, 44, 46, 56, 58 and 67, under 35 U.S.C. § 112 ¶1 has been maintained by the Examiner in view of recitation of 'molecular beacon-type probes,' and 'dual-label hydrolysis probe.' Applicants have amended the claims to obviate this issue.

The rejection of claims 27-34, 36, 38-45, 47, 49-57, 59 and 61-68 under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied in view of Wittwer et al (U.S. Patent 6,140,054, Oct. 2000) is acknowledged, and applicants have respectfully traversed this rejection, but have nonetheless amended the claims to further clarify the real-time aspect subject matter.

The rejection of claims 27-35, 38-46, 47, 49-58 and 61-67 under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied in view of Witcombe et al (U.S. Patent 6,270,967, Aug. 2001) is acknowledged, and applicants have respectfully traversed this rejection, in view of the amended claims, and further arguments.

The rejection of claims kit 61-65, under 35 U.S.C. § 103(a), as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) is acknowledged, and applicants have, to obviate this issue, amended the claims so that they are now drawn to methods using the kits.

The Examiner's rejections under obviousness-type double patenting are acknowledged, and applicants reaffirm their willingness to timely file a Terminal Disclaimer upon indication of allowable subject matter.

No new matter has been added.

35 U.S.C. § 112 ¶1 Rejections (New matter)

The Examiner has maintained the rejection of *dependent* claims 33, 35, 44, 46, 56, 58 and 67 under 35 U.S.C. § 112 ¶1, based on alleged new matter. The Examiner maintains that the specification does not describe "molecular beacon-type probes," or "dual-label hydrolysis probes" (Office Action of 28 October 2004, page 2, paragraph 5).

Applicants disagree with the Examiner's position for reasons already of record, but have nonetheless amended claims 33, 35, 44, 46, 56, 58 and 67 to delete the word 'hydrolysis,' and have further amended claims 35, 46, 58 and 67 to delete recitation of 'molecular beacons.' Additionally, claims 35, 46, 58 and 67 have been further amended to recite "nuclease cleavable dual label probe" instead of the previously recited "dual label hydrolysis probe" to further clarify the claimed subject matter. Support for recitation of "nuclease cleavable dual label probe" is found throughout the specification, and particularly at: page 9, lines 10-11; page 17, line 29; and page 21, line 21.

Applicants, therefore, respectfully request withdrawal of the Examiner's rejection of dependent claims 33, 35, 44, 46, 56, 58 and 67 under 35 U.S.C. § 112 ¶1.

35 U.S.C. § 103 Rejections

Herman in view of Wittwer:

The Examiner has maintained the rejection of claims 27-34, 36, 38-45, 47, 49-57, 59 and 61-68 under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied in view of Wittwer et al (U.S. Patent 6,140,054, Oct. 2000) (Office Action of 28 October 2004, at page 5, paragraph 7).

Specifically, the Examiner alleges that Herman teaches: "contacting the sample of genomic DNA with bisulfite"; "amplifying the converted nucleic acid with primers that distinguish between methylated and unmethylated DNA such that at least one oligonuclotide probe is a CpG specific probe [but this is incorrect, because Herman does not teach CpG probes]; and "detecting the methylated nucleic acid based on amplification mediated change or property thereof in relation to

another probe or primer" (*Id*, at pages 6-9).

The Examiner further alleges that Herman teaches "amplification is carried out using primers specific for CpG-specific oligonucleotides" (Office Action of 13 July 2004, at page 7), and "***finally*** detecting the methylated nucleic acids (citing col.5, lines 60-67) (emphasis added).

The Examiner alleges Herman teaches that amplified products are preferably identified by sequencing, and further asserts that allele-specific oligonucleotide (ASO) probe detection is among Herman et al's listed means for sequencing the amplified products.

The Examiner appreciates that the detection of Herman et al is ***subsequent*** to amplification (unlike the present invention), but the Examiner further alleges that while Herman does not teach using FRET probes to detect allele specific differences, Wittwer et al nonetheless do (Office Action of 28 October 2004 at page 7, lines 6-8). Specifically, the Examiner asserts that Wittwer teaches identification of polymorphisms using FRET probe pairs in combination with PCR and standard melting curve analysis (*Id*, at lines19-20).

According to the Examiner, it would have been *prima facie* obvious to one of ordinary skill at the time of the present invention to have modified and improved the method of Herman by using ASO probes with the allele specific detection method of Wittwer (premised on an analogy between genomic alleles and bisulfite-treated DNA) (*Id*, at page 8, lines17-20).

Applicants respectfully traverse this rejection based on the arguments of record and as extended herein. Briefly, Herman et al do not teach the required use of real-time CpG-specific probes. Rather, Herman et al teach detection *after* amplification (*i.e*, *end-point* analysis).

Likewise, while Wittwer et al may teach the use of FRET probes for allele discrimination, the Wittwer method is not a real-time method, but is at best a ***quasi*** real-time method based on post-amplification end-point analysis, because the method at its essence is based on monitoring fluorescence as a function of temperature to determine a "PCR product melting curve" (*see*, e.g., column 15, lines 55-58; column 16, lines 1-10). The "generated melting curve is ***then*** compared to the known melting curve for the mutant, normal or polymorphic sequence to determine the sequence of the target nucleic acid" (column 4, lines 14-17). Significantly, in the Wittwer method,

the FRET probe pair signal is completely abolished every time the PCR temperature is raised (dissociating and thus separating the FRET pair), and a melting curve must be determined and compared. The sensitivity of the post-amplification melting curve assay gradually increases with repeated PCR cycles as the amount of amplificate (and thus product hybridization target) increase. By constrast, the instant real-time methods to not require determination of melting curves as do the claimed methods of Wittwer et al.

Therefore, there is no teaching or suggestion in the art for *real-time* detection of methylated DNA using CpG-specific probes. In Wittwer, the PCR reaction, containing the probes (one of which is designed to be genotype-specific) is run for multiple cycles, followed by "an appended analytical cycle *after* amplification" for determining the genotype based on differences in the melting curves. The genotype determination is <u>not</u> *real-time* but occurs *after* PCR amplification during an appended analytical cycle. Thus, the alleged prior art allele discrimination methods generally involve *post-amplification melting curve analyses* (Wittwer), or are *end-point* based (Herman et al), rather than real-time, in both cases *teaching away* from the present invention.

Nonetheless, to facilitate prosecution, applicants have amended *independent* claims 27, 38 and 50 and 61 to recite "detecting *contemporaneously* with amplification."(emphasis added) to further clarify the *real-time* aspect of the presently claimed subject matter.

Support for the recitation of "detecting *contemporaneously* with amplification" is found throughout the originally-filed application, and in particular at page 12, lines 30 and 31, teaching contemporaneous (*i.e.*, real-time) detection.

Applicants, therefore, respectfully request withdrawal of the Examiner's rejection of claims 27-34, 36, 38-45, 47, 49-57, 59 and 61-68 under 35 U.S.C. § 103(a) in view of applicants' amended *independent* claims 27, 38, 50 and 61.

<u>Herman in view of Witcombe</u>:

The Examiner has maintained the rejection of claims 27-35, 38-46, 47, 49-58 and 61-67

under 35 U.S.C. § 103(a) as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000) as applied in view of Witcombe et al (U.S. Patent 6,270,967, Aug. 2001).

Specifically, the Examiner alleges that while Herman does not teach using TaqMan probes to detect allele specific differences, Witcombe et al nonetheless do (Office Action of 28 october 2004 at page 11, paragraph 8).

Applicants respectfully traverse this rejection, based on the fact that there is no teaching or suggestion in Herman et al, or in Witcombe et al to use CpG-specific probes for real-time detection of DNA methylation. Again, Herman teaches away from such an application by specifying that methylation detection is by means of CpG-specific primers, and not probes.

Significantly, and contrary to the Examiner's assertion (in reference to Figure 10, col. 8, lines, 58-62), Whitcombe does not use TaqMan (real-time probes) that are specific for allele discrimination of the ASO element. Rather, for allele discrimination of the ASO element, Whitcombe uses "tailed diagnostic primers" having a "tag region" and a "detector region" on the non-hybridizing tail, and does not use real-time TaqMan primers to directly discriminate the ASO element (see col. 1, lines 35-67 and extending to col. 2. lines 1-25). In the teachings of Whitcombe, an extension product is initially generated by extending the "tailed diagnostic primer," followed by production of a "further primer extension product" (reverse primed off the initial extension product using a "further primer." The "further primer extension product" includes (at the 3'-end thereof) a sequence complementary to the "detector region" of the tail of the tailed diagnostic primer (*Id*). Significantly, the detector region sequence is different than the ASO element being discriminated (*Id*). Various "detection probes" are discussed that recognize the detector region sequence (not the ASO allele), and include TaqMan probes (as is shown in Figure 10, cited by the Examiner). Thus, while TaqMan probes are used in the context of allele discrimination of an ASO element, such use is *indirect*, and no where does Whitcombe teach or suggest using the TaqMan or any other real-time probe for that is CpG-specific, or even ASO-specific for that matter. In fact, the "detection probes" of Whitcomb are not ASO-specific at all, and rather they non-discriminately hybridize to the "detector region" on the non-hybridizing tail of

the detection primer.

Therefore, there is no teaching or suggestion in the asserted art to combine real-time allele discrimination methods with a methylation assay that is dependent on methylation detection by means of CpG-specific probes, and not primers.

Additionally, applicants point out that *real-time* assays were in the art as early as 1995 and 1996, and MSP technology was published in 1996. Therefore, more than **four years** transpired after the advent of real-time technology, and more than **three years** transpired after the advent of MSP before the present applicants were the first to conceive of combining bisulfite treatment of DNA, methylation-specific probes and *real-time* PCR to address the long-standing need for providing efficient and quantitative real-time methylation assays, based on *methylation-sensitive probes*. There was absolutely no suggestion in the art before or during this 4-year period to make such a combination. In fact, during this period and contrary to the Examiner's assertion that "it would have been *prima facie* obvious to one of ordinary skill at the time of the present invention to have modified and improved the method of Herman by using ASO probes with the allele specific detection method of Wittwer" the *skilled* people in the art were actually *teaching away* by introducing and promoting *end-point* fluorescence assays and *fluorescent melting curves* after multiple PCR cycles, along with other solutions (not cited by the Examiner) involving the use of methylation-sensitive restriction enzymes, and primer-extension of nonmethylation-sensitive primers.

Both the lack of teachings, and the contrary teachings of the relevant art, along with the relative timing of the various inventions in the face of a substantial unmet need support applicants' contention. Significantly, clinically relevant differences in methylation are at times relatively small. The instant invention, accurately quantifies amounts of methylated amplificates, affording great diagnostic advantages, particularly where methylation differences are not large between *normal* and *abnormal/disorder* states (*e.g.*, differences of about 20-40% *normal* methylation versus an abnormal level of only about 60-80%). During the 4-year period after the separate advent of real-time technology and MSP technology the crucial need for a high-throughput and

quantitative methylation assay remained unmet, and the skilled practitioners in the relevant art were promoting methods comprising *end-point* fluorescence assays, *fluorescent melting curves*, the use of methylation-sensitive restriction enzymes, and post-amplification primer-extension

Therefore, in view of the above described claim amendments, arguments, and those already of record, applicants respectfully request withdrawal of the Examiner's rejection of claims 27-35, 38-46, 47, 49-58 and 61-67 under 35 U.S.C. § 103(a), in view of Herman et al, and further in view of Whitcombe.

Kit claims:

The Examiner has maintained the rejection of claims 61-65, under 35 U.S.C. § 103(a), as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000).

Specifically, the Examiner alleges that while Herman does not teach packaging a probe necessary for the method into a kit, the method of Herman specifically encompasses using a CpG specific probe for detection of the methylation status of the nucleic acid (Office Action of 18 October 2004, at page 17, lines 5-8).

Applicants have, to facilitate prosecution, amended independent claim 61 to recite "A method for detecting cytosine methylation within a genomic sample of DNA, comprising: obtaining a methylation kit; conducting, using the kit, a real-time methylation assay of the genomic DNA sample; and determining, based on the methylation assay, whether cytosine methylation is present in the DNA sample, and wherein the methylation kit comprises...."

Conforming amendments have been made to dependent claims 62-68, and the claims are now drawn to using the methylation assay kits in *real-time* methylation assays.

Support for these amendments is inherent to the originally filed claim set, and is as discussed herein above.

Applicants, therefore, respectfully request withdrawal of the rejection of claims 61-65, under 35 U.S.C. § 103(a), as being unpatentable over Herman et al. (U.S. Patent 6,017,704, Jan. 25, 2000).

Obviousness-type Double Patenting Rejection

The Examiners has maintained the rejection of claims 27-32, 38-43, 50-55 and 61-67 under the judicially created doctrine of obviousness-type double patenting as being unpatentable in view of claims 1-26 of U.S. Patent No. 6,331,393 (December 18, 2001) (Office Action of 28 October 2004, at page 18).

Applicants are fully prepared to timely file a Terminal Disclaimer upon notification of allowable subject matter.

Applicants respectfully request reconsideration and allowance of all pending claims 27-36, 38-47, 49-59 and 61-68 of the present application.

No new matter has been added.

Entry of the Amendment is respectfully requested.

Respectfully submitted,

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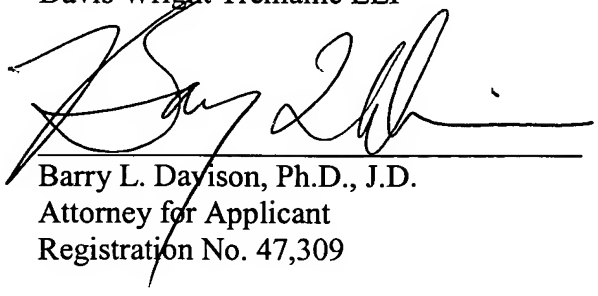

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